ABSTRACT

Methods for in vitro genotyping of drug-sensitive and drug-resistant subsets of primary tumors.

Drug discovery methods have evolved from empirical screening models to targeted therapeutics. However, the complex genomic response to rationally designed agents is beyond the horizon of most screening processes. We are developing methods to characterize the genomic response of primary tumors to various classes of anti-tumor agents. Our focus has been on the selection of malignant cells from the tumor background after in vitro drug exposure in order to clarify the source of mRNA signals detected on gene arrays. Towards this end, we developed cell-sorting techniques that enable us to purify tumor cells from the stromal background using flow cytometry. Freshly resected tumors are initially processed into fixed sections and cell suspensions. Suspensions are cultured in non-adherent cytophobic plates in the presence of selected anticancer agents for varying lengths of time. Tissue sections are analyzed by immunohistochemical staining with various monoclonal antibodies that identify tumor-specific markers. At set intervals, tissue culture aliquots are removed from the drug exposure environment, washed, and stained with fluorescently conjugated antibodies for flow cytometric analysis. Cells are also labeled with annexin V to identify cells undergoing apoptosis. Based on the specificity of double labeling for malignant cells and cells undergoing apoptosis, two populations of malignant cells are sorted: drug sensitive and drug resistant. Sorting also serves to eliminate the non-malignant components present in the primary tissue culture. Sensitive and resistant cells from the same genetic background are then compared by extracting and amplifying mRNA, which is evaluated by micro-array display. Kinetics of mRNA expression after drug exposure are evaluated and informatics studies relating these changes to drug sensitivity or resistance are carried out.